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Review

Early-phase neuroplasticity induced by offline transcranial ultrasound stimulation in primates☆

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The use of 'offline' transcranial ultrasound stimulation (TUS) protocols is of particular interest in the rapidly growing field of low-intensity TUS. Offline TUS can modulate neural activity up to several hours after stimulation, suggesting the induction of early-phase neuroplasticity. Studies in both humans and nonhuman primates have shown spatially specific changes in both the neuromodulation target and in a distributed network of regions associated with it. These changes suggest that excitatory or inhibitory effects are a result of a complex interaction between the protocol used and the underlying brain region and state. Understanding how early-phase neuroplasticity is induced by offline TUS could open avenues for influencing late-phase neuroplasticity and therapeutic applications in a wide range of brain disorders.

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Introduction

Adaptive neuroplasticity is integral both in correcting or improving aberrant function in a large range of neurological and psychiatric disorders and in ensuring normal functioning in healthy ageing. Measuring and inducing neuroplasticity can thus be beneficial therapeutically and can also improve our understanding of the brain in general health [1]. Brain stimulation, or more generally, neuromodulation methods, can elicit functional brain changes and thereby promote neuroplasticity [2]. Recently, transcranial ultrasound stimulation (TUS) applied at low intensity has been found to safely induce neuronal changes with high spatial specificity in both superficial cortex and deep brain regions [3,4] when careful considerations are taken to limit the transmission loss caused by the skull [5]. TUS uses acoustic energy to mainly leverage the mechanosensitivity of neural tissue to bring about changes in neuroplasticity [6,7].

TUS protocols can be categorised into online or offline according to the duration of their effects. The term 'online' refers to TUS protocols aimed at triggering acute effects, which occur only during or immediately after the neuromodulation period. These online interventions, particularly in humans, usually involve pulse trains that do not last more than half a second (see Figure 1a and legend for TUS parameter definition). These are hypothesised to change the underlying brain activity of the region targeted, with some evidence for network changes but produce no lasting effects beyond the stimulation period itself. 'Offline' TUS protocols on the other hand aim to induce effects that significantly outlast the stimulation period, for example, by minutes or hours, even days, after the intervention. These protocols are usually characterised by long duration pulses or trains of pulses that typically last 20 s or longer. These are thought to induce both local and distributed changes across the whole brain. There is an ongoing debate in the TUS community about the presence of sensory costimulation (e.g. the sound that accompanies TUS protocols) and how this may influence the effects of TUS [8]. While the delayed readout in offline studies reduces the risk of such issues, placebo/nocebo effects could still be based on both participants' and researchers' expectations. These issues can be mitigated by introducing good study control and double-blinding procedures (see Table 1).

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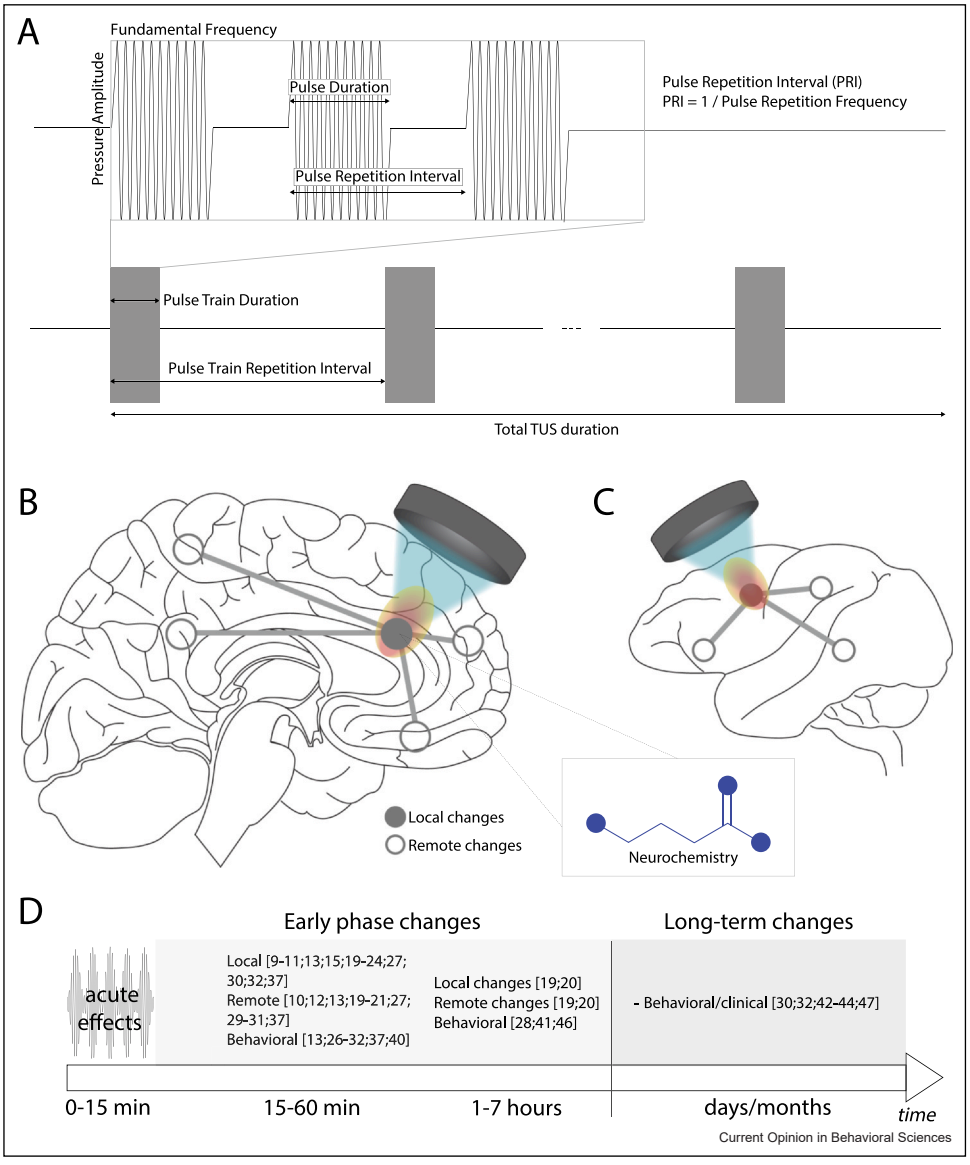
Figure 1



Schematic of an ultrasound pressure waveform and illustrations of some of the offline TUS effects presented in this review. **(a)** A rectangular ramp shape is used to present the pressure amplitude. Typically, the positive and negative amplitudes are the same when operating at low pressures, to be within the linear regime. A single continuous sonication is a pulse and has a duration of PD. Pulses are often repeated in a pulse train. The duration between two pulses in a pulse train is the pulse repetition interval and is equal to 1 divided by the PRF. The pulse train will have a duration, which is the pulse train duration. The pulse train can be repeated and, if so, has a structure similar to the pulse. **(b)** Local and remote effects in humans, including changes in neurochemistry. **(c)** Local and remote effects in nonhuman primates. **(d)** Summary of the timescale of effects following offline TUS.

In this review, we will explore the ability of offline TUS, in primates, to induce changes in behaviour and neural activity that outlast the sonication period by minutes, hours, or even days, covering papers up to August 2023. We will then discuss whether the observed effects can be related to changes in synaptic strength (i.e. synaptic plasticity) and induction of long-term effects. This is crucial to lay the groundwork for translating offline TUS protocols into clinical interventions in which TUS will produce long-lasting therapeutic changes.

Measuring TUS-induced neuroplasticity

The effects mediated by offline TUS include both local and remote neuronal changes (Figure 1a and b) as well as changes in behaviour related to specific cognitive engagement and in their associated neural correlates. These changes occur in the minutes, hours, or days following TUS (Figure 1c). These changes could be induced when subjects are at rest or under anaesthesia or could be evoked by using another brain stimulation method or by engaging in a behavioural task. The same

Table 1

Summary of offline TUS protocols used in primate studies included in this review.

Paper	Species	Condition, state	Readout	Brain area	Transducer placement	Evaluation of stimulation efficacy/ target engagement	Controls	Blinding	TUS protocol: FF; PD; PRF; duration; repetition (if any)
Monti et al., 2016 [43]	Human	Disorder of consciousness, not awake	Behaviour	Thalamus	MR guided	No	No	P blind state R not blind	FF = 650 kHz; PD = 0.5 ms; PRF = 100 Hz; duration = 30 s; Repeated every 30 s over a period of 10 min
Badran et al., 2020 [40]	Human	Healthy, awake	fMRI	Thalamus	MR guided	No	No stim.	P blind– R blind collection and analyses	FF = 650 kHz; PD = 5 ms; PRF = 10 Hz; duration = 30 s; Repeated every 30 s over a period of 10 min
Reznik et al., 2020 [42]	Human	Depression, awake	Behaviour	Frontotemporal	Not reported	k-Wave and example CT	No stim.	P blind– R blind collection	FF = 500 kHz; PD = 0.065 ms; PRF = 40 Hz; duration = 30 s
Sanguinetti et al., 2020 [41]	Human	Healthy, awake	fMRI	Inferior frontal gyrus	Localiser cap	k-Wave and example CT	No stim.	P blind– R blind collection	FF = 500 kHz; PD = 0.065 ms; PRF = 40 Hz; duration = 30 s
Cain et al., 2021 [12]	Human	Healthy, awake	fMRI; ASL	Globus pallidus	MR guided	k-Wave and example CT	No	P not blind R not blind	*Low PRF protocol:* FF = 650 kHz; PD = 5 ms; PRF = 10 Hz; duration = 30 s; Repeated every 30 s for 10 min; *High PRF protocol:* FF = 650 kHz; PD = 0.5 ms; PRF = 100 Hz; duration = 30 s; Repeated every 30 s for 10 min
Cain et al., 2021 [44]	Human	Disorder of consciousness, not awake	Behaviour	Thalamus	MR guided	No	No	P blind state R not blind	FF = 650 kHz; PD = 0.5 ms; PRF = 100 Hz; duration = 30 s; Repeated every 30 s for 10 min
Zhang et al., 2021 [22]	Human	Healthy, awake	TMS; behaviour	M1 hand	TMS-MEP hotspot	k-Wave and example anat.	No stim.	P blind– R not blind	FF = 500 kHz; PD = 500 μs; PRF = 100 Hz; duration = 500 ms; Repeated every 8 s for 15 min
Lee et al., 2022 [47]	Human	Epilepsy, awake	Stereo EEG; seizure monitoring	Seizure onset zone	MR guided	k-Wave and indiv. CT	No stim. Photic stim	P blind R not blind	FF = not reported; PD = 3 ms; PRF = 100 Hz; duration = 10 min
Nakajima et al., 2022 [37]	Human	Healthy, awake	fMRI; behaviour	M1 hand, STN, putamen, aIFC, middle frontal cortex	Neuronav. indiv. struct. MRI	k-Wave and indiv. struct. MRI	Active in study 3 only	P not blind R not blind	FF = 500 kHz; PD = 30 ms; PRF = 10 Hz; duration = 40 s
Samuel et al., 2022 [16]	Human	Healthy, awake	TMS; MEG	M1 hand	TMS-MEP hotspot	No	No stim.	P not blind R not blind	FF = 500 kHz; PD = 20 ms; PRF = 5 Hz; duration = 80 s
Zeng et al., 2022 [23]	Human	Healthy, awake	TMS	M1 hand	TMS-MEP hotspot	No	Sham and active	P not blind R not blind	*Low PRF protocol:* FF = 500 kHz; PD = 20 ms; PRF = 5 Hz; duration = 80 s *High PRF protocol:* FF = 500 kHz; PD = 0.32 ms; PRF = 1000 Hz; duration = 80 s; Repeated every 1.1 s for 55 s

Table 1 (*continued*)

Paper	Species	Condition, state	Readout	Brain area	Transducer placement	Evaluation of stimulation efficacy/ target engagement	Controls	Blinding	TUS protocol: FF; PD; PRF; duration; repetition (if any)
Chou et al., 2023[a]	Human	Healthy, awake	fMRI; pain response	Amygdala	Not reported	No	No stim.	P not blind R not blind	Not reported
Kuhn et al., 2023 [13]	Human	Healthy, awake	fMRI; ASL	Amygdala, entorhinal cortex	MR guided	Prior water tank measurements and skull	Active	P blind+ R blind collection and analyses	*Amygdala protocol:* FF = 650 kHz; PD = 5 ms; PRF = 10 Hz; duration = 30 s; Repeated every 30 s for 5 min *Entorhinal cortex protocol:* FF = 650 kHz; PD = 0.5 ms; PRF = 100 Hz; duration = 30 s; Repeated every 30 s for 5 min
Ren et al., 2023 [24]	Human	Healthy, awake	TMS	M1 hand	Localiser cap	No	No stim.	P not blind R not blind	FF = 500 kHz; PD = 500 μs; PRF = 100 Hz; duration = 500 ms Repeated every 8 s for 15 min
Yaakub et al., 2023 [10]	Human	Healthy, awake	fMRI; MRS	PCC, dACC	Neuronav. indiv. struct. MRI	k-Wave and indiv. struct. MRI	Sham and active	P blind+ R not blind	FF = 500 kHz; PD = 20 ms; PRF = 5 Hz; duration = 80 s
Zhang et al., 2023 [11]	Human	Healthy, awake	TMS; MRS	M1 hand	Neuronav. indiv. struct. MRI	Water tank measurements and skull Onscale and example CTs	No stim. in study 1 No controls in studies 2 and 3	P not blind R not blind	*Low PRF protocol:* FF = 500 kHz; PD = 400 μs; PRF = 50 Hz; duration = 500 ms; Repeated every 2 s for 5 min *High PRF protocol:* FF = 500 kHz; PD = 200 μs; PRF = 2000 Hz; duration = 500 ms; Repeated every 2 s for 5 min
Verhagen et al., 2019 [19]	Rhesus macaque	Healthy, sedated	fMRI	Frontal polar cortex	Neuronav. indiv. struct. MRI	Custom and example CT	No stim. and active	P blind state R not blind	FF = 250 kHz; PD = 30 ms; PRF = 10 Hz; duration = 40 s
Folloni et al., 2019 [20]	Rhesus macaque	Healthy, sedated	fMRI	pACC; amygdala	Neuronav. indiv. struct. MRI	Custom and example CT	No stim. and active	P blind state R not blind	FF = 250 kHz; PD = 30 ms; PRF = 10 Hz; duration = 40 s
Fouragnan et al., 2019 [28]	Rhesus macaque	Healthy, awake	fMRI	pACC	Neuronav. indiv. struct. MRI	Custom and example CT	No stim. and active	P blind state R not blind	FF = 250 kHz; PD = 30 ms; PRF = 10 Hz; duration = 40 s
Khalighinejad et al., 2020 [29]	Rhesus macaque	Healthy, sedated	fMRI	Basal forebrain	Neuronav. indiv. struct. MRI	Custom and example CT	No stim. and active	P blind state R not blind	FF = 250 kHz; PD = 30 ms; PRF = 10 Hz; duration = 40 s
Pouget et al., 2020 [31]	Rhesus macaque	Healthy, awake	Behaviour	FEF	Neuronav. indiv. struct. MRI	k-Wave and example CT	No stim. and active	P blind state R not blind	FF = 320 kHz; PD = 30 ms; PRF = 10 Hz; duration = 20 s
Zou et al., 2020 [46]	Rhesus macaque	Epilepsy model, awake	Video EEG	M1 hand	Not reported	Water tank measurements and skull	No stim.	P blind state R not blind	FF = 800 kHz; PD = 0.555 ms; PRF = 500 kHz; duration = 15 min
Bongioanni et al., 2021 [26]	Rhesus macaque	Healthy, awake	Behaviour	Medial frontal cortex	Neuronav. indiv. struct. MRI	No	No stim. and active	P blind state R not blind	FF = 250 kHz; PD = 30 ms; PRF = 10 Hz; duration = 40 s

Table 1 (*continued*)

Paper	Species	Condition, state	Readout	Brain area	Transducer placement	Evaluation of stimulation efficacy/ target engagement	Controls	Blinding	TUS protocol: FF; PD; PRF; duration; repetition (if any)
Folloni et al., 2021 [27]	Rhesus macaque	Healthy, awake	fMRI; behaviour	Lateral OFC; anterior PFC	Neuronav. indiv. struct. MRI	Custom and example CT	No stim. and active	P blind state R not blind	FF = 250 kHz; PD = 30 ms; PRF = 10 Hz; duration = 40 s
Munoz et al., 2022 [30]	Rhesus macaque	Healthy, awake	fMRI	Striatum	Neuronav. indiv. struct. MRI	No	No stim. and active	P blind state R not blind	FF = 500 kHz; PD = 10 ms; PRF = 2 Hz; duration = 2 min
Liu et al., 2023 [21]	Nonhuman primate (unspecified)	Healthy, sedated	fMRI	Caudate nucleus	Neuronav. template	k-Wave and example CT	No stim.	P blind state R not blind	FF = 500 kHz; PD = 10 ms; PRF = 2 Hz; duration = 2 min
Webb et al., 2023 [32]	Rhesus macaque	Healthy, awake	Intracranial EEG; behaviour	LGN	Custom head frame	MR thermometry	Sham and active	P blind state R not blind	FF = 480 kHz; PD = 30 ms; PRF = variable; duration = 30 s; Repeated daily for more than 6 months

Brain area: M1, primary motor cortex; aIFC, anterior inferior frontal cortex; dACC, dorsal ACC; pACC, perigenual ACC; FEF, frontal eye field; OFC, orbitofrontal cortex.
Transducer placement: 'Neuronav. indiv. struct MRI', stereotaxic neuronavigation system, tracked continuously with infrared reflectors, using participant's MRI structural (T1-weighted) image. 'Neuronav. template', example stereotaxic neuronavigation system, tracked continuously with infrared reflectors, using a template MRI structural image. 'MR guided', iterative transducer placement using MRI of the transducer position on scalp. 'Localiser cap', transducer positioned using electrode position on EEG cap or scalp distance measurements. 'TMS-MEP hotspot', point on scalp associated with highest MEP after TMS stimulation.
Evaluation of stimulation efficacy/target engagement: 'example CT/example anat.', simulation run using a template/example CT scan(s) or an example MRI anatomical image(s) converted into a pseudo-CT(s). 'k-Wave/custom indiv. CT/struct. MRI', individual simulations run with k-wave or custom scripts, using the participant's CT or MRI anatomical image converted into a pseudo-CT. 'Water tank measurements & skull', water tank measurements using a hydrophone and *ex vivo* skull in degassed water.
Controls: 'Active', active control brain region. 'Sham', sham control including sound control (unfocused stimulation, sound masking, white noise, or replicating stimulation sound using bone-conducting headphones). 'No stim.', control session without simulation or sham control excluding sound control. No, no control session/group.
Blinding: 'P blind+', participant blinding with successful postcheck. 'P blind–', participant blinding attempted with unsuccessful postcheck or failure to control for sound. 'P not blind', participant not blinded or blinding procedure not reported. 'P blind state', participant is nonhuman primate or human in a minimally conscious state. 'R blind collection', researcher blinding during data collection. 'R blind collection and analyses', researcher blinding during data collection and analyses. 'R not blind', researcher not blinded or blinding procedure not reported.
TUS protocol: FF, fundamental frequency; PD, pulse duration.
[a] Abstract 38 in *Biological Psychiatry* 2023, 93:S84–S85.

effects have also been investigated with online TUS (for a review on online TUS effects, please see Refs. [7,9]).

Taking advantage of their high spatial specificity, a range of noninvasive neuroimaging methods have been used to assess local and remote neuronal effects of offline TUS in primates (Figure 1a and b). These effects include, but are not limited to, local and global changes in blood flow–related brain activity and connectivity (task-based and resting-state functional magnetic resonance imaging [fMRI]), metabolite concentrations (magnetic resonance spectroscopy [MRS]), and perfusion (arterial spin labelling [ASL]). With higher temporal resolution, magnetoencephalography (MEG) and electroencephalography (EEG) allow the investigation of time–frequency–dependent brain activity, and the temporal dynamics of changes in brain oscillations induced by TUS. Offline TUS can also be coupled with other forms of brain stimulation, such as transcranial magnetic stimulation (TMS) to modulate another form of evoked response, for example, motor evoked potentials (MEP) induced by TMS.

In the absence of neuroimaging or additional neurostimulation methods to quantify measures of neuroplasticity, TUS can simply be used to impact behavioural or cognitive functioning, arguably the ultimate output of network activity. However, unlike other brain stimulation methods, there is currently no evidence that TUS can elicit readily observable behavioural readouts that can confirm target engagement (e.g. a finger twitch, as elicited by TMS of the hand area of the motor cortex). With higher order cognition, behavioural readout becomes less informative regarding TUS target engagement. In these cases, some other method for inferring target engagement can be useful, for example, through acoustic simulations.

Evidence supporting local changes

Techniques for investigating the local impact of offline TUS include assessing changes within the anatomically defined brain region at the location of the peak intensity of TUS or within specified boundaries of the acoustic pressure field of the TUS. Two human studies used MRS to measure the concentration of γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter, in a voxel broadly overlapping with the TUS focal pressure field [10,11]. Using a low pulse repetition frequency (PRF) TUS protocol (5 Hz) targeted on the posterior cingulate cortex (PCC; see Table 1 for details on all protocols), Yaakub et al. observed a decrease in the concentration of GABA in the PCC but not in the dorsal anterior cingulate cortex (ACC), indicating a spatially specific increase in excitability in the hour following TUS [10]. Revealing longer lasting effects, Zhang et al. [11] found that both excitatory and inhibitory effects of TUS on GABA levels in the motor cortex depend on the type of TUS protocol applied. With repeated sonication over 7 days, the effects can persist for up to 24 hours. Using ASL, one study [12] found a decrease in perfusion, indicating inhibition, after stimulating the basal ganglia, while another [13] reported an increase in perfusion following amygdala and entorhinal cortex stimulation. These seemingly contradictory inhibitory and excitatory effects may indicate complex relationships between TUS protocols, tissue composition, and states [14,15].

Evidence supporting spatial specificity of distributed network changes

The effects of offline TUS can be observed not just locally, at the site of stimulation, but in a network of regions associated with the stimulation site. Using a low PRF protocol (5 Hz), TUS applied to the human motor cortex has been found to not only change MEG alpha power in the motor cortex and increase local MEG connectivity within the motor areas but also affect beta power in functionally connected regions up to 25 min post-TUS [16]. Confirming the intricate offline TUS impact on brain networks, multiple nonhuman primate studies found significant changes in coupling between the sonicated region and its functionally relevant neural network or 'connectivity fingerprint' [17,18]. These studies showed that TUS of specific deep cortical and subcortical regions, while the animals were under anaesthesia, perturbed the connectivity profile of the sonicated region up to 2 hours after TUS. These effects were regionally specific: sonication of distinct regions of the medial frontal cortex caused changes in each area's connectivity fingerprint only when TUS was applied to the area itself, and not to a control region [19], even when the two brain areas are only a few millimetres apart [20]. This was confirmed in another nonhuman primate study targeting the caudate nucleus with a lower PRF protocol [21] (2 Hz PRF instead of 10 Hz PRF in the previous two studies [19,20]). In humans, the same was observed after TUS of the dorsal ACC and PCC were performed [10], with these changes showing a time dependence where functional connectivity of the target region was initially limited to a small network of regions during the early fMRI acquisition (at approximately 13 min post-TUS), with later changes (at approximately 46 min post-TUS) involving a larger network of regions.

Modulation of evoked response

Neuroplasticity induced by TUS may also include excitability or inhibitory effects that can be measured with TMS (e.g. TMS-induced MEP). In a series of work combining offline TUS and TMS in humans, MEP amplitudes were amplified by repetitive low PRF offline TUS (PRF range 5–100 Hz; see Table 1 for details of the protocols) [22–24]. Facilitatory effects were still present 30 min postsonication in one study [24], confirming the duration of offline TUS effects on neural transmission.

Contrasting with these results, Zhang et al. [11] found excitatory effects in the form of decreased intracortical inhibition produced by a high PRF protocol (2000 Hz) and inhibitory effects — reduced MEP amplitudes, increased intracortical inhibition and decreased intracortical facilitation — with a lower PRF protocol (50 Hz). Drawing a conclusion about the impact of PRF on excitation and inhibition from these studies is difficult as other TUS parameters also differed between the studies. Furthermore, the latter study [11] was the only one (of the four reported here) to use stereotaxic neuronavigation and acoustic simulation to ensure more accurate and efficient targeting of M1. As the effect of specific protocol parameters on the type and duration of offline effects is still poorly understood, the field would benefit from further systematic exploration of the parameter space.

Nonhuman primates: task-related changes and associated brain networks

The ability of TUS to induce neuronal changes in the targeted region and its associated network that outlast the stimulation itself can open vast avenues to elucidate brain–behaviour relationships. As such, offline TUS can be used to modulate performance during a cognitive task following TUS intervention [25]. In nonhuman primates, a low PRF protocol (10 Hz) has been found to perturb activity in specific parts of the frontal cortex [26–28] and basal forebrain [29], but not adjacent brain regions. This manipulation had direct effects on behaviour, revealing the causal role of the perigenual ACC in translating cue information into choices [28], of the area 47/12o in credit assignment [27], of the basal forebrain in altering the timing of decisions [29] and of the medial frontal cortex in estimating novel choice values [26]. Another low PRF offline protocol (2 Hz) also modified motivational and cognitive aspects of behavioural performance in a motivated decision-making task [30].

In addition to perturbing high-level decision-making processes, offline TUS can interfere with perceptual processes. Using a saccade task, TUS directed to oculomotor regions perturbed saccade latencies up to 20 min postsonication [31]. During a visual discrimination task, offline TUS applied to the lateral geniculate nucleus (LGN) produced a choice bias towards the contralateral hemifield peaking 15 min after TUS with an increase in gamma activity measured with intracranial EEG. Surprisingly, and questioning the possibility for longer-term changes in neuroplasticity, the TUS-induced bias reduced over the course of 5 months of daily sessions due to adaptation, although the effect did reappear after the first TUS session following a 1-month break [32].

Task-related changes in humans

The large majority of human studies targeting task-dependent cognitive processes make use of online TUS protocols to find acute TUS-evoked effects [8,33–36]. Nevertheless, the efficacy of offline stimulation in changing behaviour over time and beyond the stimulation period itself is beginning to be established. Offline TUS of the anterior putamen, subthalamic nucleus (STN), and inferior frontal cortex caused a sustained disruption of motor response inhibition during a stop-signal task, effective for several minutes after sonication [37]. It is noteworthy that the behavioural changes that resulted from disruption of STN activity mirrored those observed in deep brain stimulation [38] and lesion studies [39]. Badran et al. [40] reported an attenuation of thermal pain sensitivity in the 10 min following an offline TUS protocol targeting the thalamus. Additional studies have linked changes in the activity of specific brain regions induced by offline TUS to variations in affect and mood [41] (and Chou et al., abstract 38 in *Biological Psychiatry* 2023, 93:S84–S85). It should be noted that offline TUS research in humans is still in its early stages and thus a large variability exists across studies. Factors such as stimulation parameters, control and blinding procedures, safety and transcranial acoustic simulations should be taken into consideration when interpreting study findings.

Clinical applications

The therapeutic potential of TUS lies in its ability to generate long-lasting changes both at the neural and behavioural levels, possibly after repeated interventions. Following the initial finding of a positive effect on mood after dorsolateral prefrontal cortex (PFC) sonication [41], the same group conducted a preclinical study with depressed participants [42]. Replicating their previous findings, they found that global affect increased over the course of the 5-day TUS intervention. However, this effect did not persist when assessed at a 1-month follow-up.

Long-term results were obtained with patients who partially recovered from a minimally conscious state after receiving thalamic ultrasound stimulation [43,44]. One patient who received ten 30-second sonications 19 days postinjury showed gradual signs of recovery starting from the day after the intervention [43]. Two of three patients in a long-lasting minimally conscious state improved after receiving two thalamic stimulation sessions of 10 min each [44]. Along with the reported antinociceptive effects of thalamic ultrasound stimulation described earlier [40], these results are encouraging for the pursuit of long-term, stable, plastic neuronal reconfiguration.

There are ongoing clinical trials testing the efficacy of TUS for the treatment of drug-resistant epilepsy [45] after its safety has been established in animal models. In a penicillin-induced epilepsy model in two nonhuman primates, offline TUS decreased the seizure frequency

and duration in both macaques for up to 7 hours after TUS [46]. A pilot study in patients with drug-resistant epilepsy [47] reported a decrease in seizure frequency in two of three patients in the 2 days following TUS of the seizure onset zone. Intracranial EEG recordings at the seizure onset zone revealed an increase in spectral power during sonication, followed by a decrease in power, for several patients. Although the authors failed to identify a link between the two measures and despite the variable response to TUS treatment, these observations also support the potential for TUS to induce neuronal plasticity.

Discussion on plasticity

The range of effects that has been presented in this review suggests that offline TUS can elicit long-term potentiation (LTP)–like plasticity, modifying neural circuits up to several hours after intervention. Several *in vivo* studies in small animals provide evidence that TUS may trigger long-lasting activity-dependent synaptic modifications through LTP and long-term depolarisation [48,49]. At the neuronal level, TUS depolarises postsynaptic neurons by activating mechanosensitive, voltage-gated sodium and calcium channels, allowing calcium influx through N-methyl-D-aspartate (NMDA) receptors [6]. This increase in postsynaptic calcium level is thought to be a key requirement for triggering changes in synaptic signalling, particularly through LTP [50]. TUS was able to restore LTP and memory in ageing mice, confirming that it can modulate NMDA receptor function [48].

With repeated treatment, offline TUS has the potential to induce long-lasting functional changes, enabling its use in clinical settings. Repetitive treatment and refinement of the dose–response relationship to induce longer-term neuroplasticity has been shown to be clinically effective in other neuromodulation techniques, such as intermittent theta-burst TMS in treatment-resistant depression [51,52]. To date, the evidence supporting long-lasting changes induced by offline TUS in primates is very limited. Of the studies we reviewed, only four found behavioural changes that persisted for several days or weeks [30,32,40,44], and in some cases, the clinical benefits later vanished [44], or in other cases, the effect of the daily stimulation decreased possibly due to adaptation [32]. Nonetheless, several studies in rodents were able to produce long-lasting behavioural changes, up to weeks, after repeated TUS treatment [48,53–55]. Combined with observations *in vitro*, several hypotheses regarding the downstream signalling pathway have been tested. The proposed mechanisms include (1) the action of TUS on astrocytes — mediating the synthesis and release of neurotrophic factors, such as brain-derived neurotrophic factor, (2) neurogenesis — through the action of TUS on stem cells, as well as (3) disruption of the extracellular matrix enabling synaptic reconfiguration (for a review, see Ref. [9]). Despite the recent findings in small animal studies, translating this work from rodents to primates proves to be a challenging task. Apart from differences in brain anatomy and function, the brain size, skull thickness and stimulation parameters typically employed make it difficult to compare the effectiveness of TUS between species.

Conclusions

There is some evidence that offline TUS can induce changes up to several hours after stimulation, suggesting mediation of early-phase neuroplasticity in primates. However, it remains to be understood how offline TUS — where the duration of sonication is relatively short (in the order of tens of seconds) — can lead to seemingly persistent neuronal changes in humans. It is also crucial to widen our understanding of the impact of multiple offline TUS sessions over the course of multiple weeks in terms of safety and efficacy. Therefore, understanding the neuronal reconfiguration generated by offline TUS in humans and the impact of repeated TUS interventions should be the focus of future studies to characterise its mechanism of action.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare no conflict of interest.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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This paper reported an absence of tissue damage after low- and high-dose ultrasound in rhesus macaque and Dorset sheep. This is an important paper to demonstrate the safety of repeated TUS.

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